Foraging behaviour at the fourth trophic level: a comparative study of host location in aphid hyperparasitoids

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Abstract

In studies of foraging behaviour in a multitrophic context, the fourth trophic level has generally been ignored. We used four aphid hyperparasitoid species: *Dendrocerus carpenteri* (Curtis) (Hymenoptera: Megaspilidae), *Asaphes suspensus* Walker (Hymenoptera: Pteromalidae), *Alloxysta victrix* (Westwood) (Hymenoptera: Alloxystidae) and *Syrphophagus aphidivorus* (Mayr) (Hymenoptera: Encyrtidae), to correlate their response to different cues with their ecological attributes such as host range and host stage. In addition, we compared our results with studies of primary parasitoids on the same plant–herbivore system. First, the olfactory response of females was tested in a Y-tube olfactometer (single choice: plant, aphid, honeydew, parasitised aphid, aphid mummy, or virgin female parasitoid; dual choice: clean plant, plant with aphids, or plant–host complex). Second, their foraging behaviour was described on plants with different stimuli (honeydew, aphids, parasitised aphids, and aphid mummies). The results indicated that olfactory cues are probably not essential cues for hyperparasitoid females. In foraging behaviour on the plant, all species prolonged their total visit time and search time as compared to the control treatment (clean plant). Only *A. victrix* did not react to the honeydew. Oviposition in mummies prolonged the total visit time because of the long handling time, but the effect of this behaviour on search time could not be determined. No clear correlation between foraging behaviour and host stage or host range was found. In contrast to specialised primary aphid parasitoids that have strong fixed responses to specific kairomones and herbivore-induced synomones, more generalist aphid hyperparasitoids seem to depend less on volatile olfactory stimuli, but show similarities with primary parasitoids in their use of contact cues while searching on a plant.

Introduction

In the last two decades, much interest has been shown in the foraging behaviour of natural enemies in a multitrophic context. Insect parasitoids are known to be influenced by cues from different trophic levels to find their herbivore hosts. Among these cues are plant volatiles, herbivore induced volatiles, and direct and indirect signals from the hosts (Vet et al., 1995; Vinson, 1998). Their strategy is to zoom in on long distance cues, thereby slowly confining their search area, and then shifting from long range cues to short range cues. Within this transition we usually observe a shift from indirect, often unreliable cues, such as plant cues, to more direct and reliable cues, such as contact chemicals derived directly from the host itself, thereby increasing the probability of locating the host (Vet et al., 2002).

Parasitoids attacking herbivores are not necessarily the highest trophic level of vertical foodwebs. In many systems

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one or more higher trophic levels may exploit the parasitoids, for example hymenopterous hyperparasitoids. Although the degree of similarity between primary and secondary (or hyper-) parasitoids is obvious because of their common evolutionary origins and life-history strategies, hyperparasitoids are likely to possess specific biological attributes which enable them to exploit resources from the third trophic level (Brodeur, 2000). To find their host, hyperparasitoids can potentially make use of cues from all trophic levels. However, we have as yet gained very little insight into the chemical ecology of hyperparasitoids.

The present study explores the searching behaviour of different hyperparasitoid species and makes comparisons with the behaviour of primary parasitoids. Aphid hyperparasitoids are an ideal model, as their biology is relatively well known and they cover a great diversity of species with different life histories and host ranges (Sullivan, 1987; Sullivan & Völkl, 1999). Using a comparative approach, we studied the host search behaviour of four obligate hymenopterous hyperparasitoid species from four different families. *Dendrocerus carpenteri* (Curtis) (Megaspilidae) and *Asaphes suspensus* Walker (Pteromalidae) are generalist ectophagous idiobiont hyperparasitoids that attack the prepupa or the pupa of the primary parasitoid after it has killed and mummified the aphid (mummy host). In contrast, *Alloxysta victrix* (Westwood) (Alloxystidae) is an endophagous koinobiont hyperparasitoid that lays an egg in the parasitoid larva in the still-living aphid (parasitised aphid host), where it remains, to hatch only after mummification of the aphid. The host range of alloxistid hyperparasitoids is more restricted than that of idiobiont hyperparasitoids (van den Bosch, 1981; Sullivan & Völkl, 1999; Brodeur, 2000). Finally, *Syrphophagus aphidivorus* (Mayr) (Encyrtidae) is also an endophagous koinobiont, but it has a dual oviposition behaviour. It attacks both parasitoid larvae in live aphids and parasitoid prepupae or pupae in mummified aphids. The latter are preferred, as they are more suitable hosts for development (Kanuck & Sullivan, 1992; Buitenhuis et al., 2004a). Encyrtid hyperparasitoids have been reported to attack many different parasitoids of aphids (Aphididae) and even psyllids (Psyllidae) (Hoffer & Stary, 1970).

We tested if we could find support for the prediction that the relatively host-specific alloxystid hyperparasitoid uses general cues associated with aphids (aphids and honeydew), and specific cues from primary parasitoid females and/or host plant volatiles from the specific plant–aphid–host system (Sullivan & Völkl, 1999) to locate their host. By contrast, ecto-hyperparasitoids with a broad host range are predicted to rely less on specific cues, and to use general cues associated with aphids (aphids and honeydew) and aphid mummies on different plant–aphid–host systems (Sullivan & Völkl, 1999). The species with a dual oviposition behaviour, *S. aphidivorus*, is predicted to resemble the ecto-hyperparasitoids because of its broad host range and its preference for mummified aphids.

We focussed on two components of foraging behaviour, attraction by olfactory stimuli and behavioural response to contact stimuli on a plant. The use of olfaction by aphid hyperparasitoids was studied by testing different potentially attractive odours in a Y-tube olfactometer. Odours from all trophic levels were included, such as plant, aphid, female parasitoid, parasitised aphid, and mummified aphid odours, as well as the aphid faecal waste product, honeydew. Furthermore, plant odours possibly induced by aphids and the attraction of the whole plant–aphid–host complex were tested. A second experiment tested the influence of different short-distance cues such as honeydew, aphids, parasitised aphids, and mummified aphids on the search behaviour of hyperparasitoids. The behaviour of females was observed while they were searching on a plant that had been treated with one or a combination of these cues.

Materials and methods

Insect material

Colonies of the four hyperparasitoid species were established on the primary parasitoid *Aphidius nigripes* Ashmead (Hymenoptera: Braconidae). This parasitoid was reared on the potato aphid *Macrosiphum euphorbiae* (Thomas), on potato seedlings, *Solanum tuberosum* L. cv. Norland following the techniques of Brodeur & McNeil (1994a). All four hyperparasitoids have been reported in the field on this experimental system in North America (Shands et al., 1965; Brodeur & McNeil, 1994b). The hyperparasitoid *A. victrix* originated from a laboratory strain in Newport, UK, *A. suspensus* from a field population in Quebec, Canada, *D. carpenteri* from a laboratory strain in Burnaby, Canada, and *S. aphidivorus* from a laboratory strain in Bayreuth, Germany. All insects had been held in the laboratory for more than 10 generations before being used in the experiments.

Hyperparasitoid colonies were maintained by exposing potato plants, which had been infested with mummified aphids (for *A. suspensus*, *D. carpenteri*, and *S. aphidivorus*) or live parasitised aphids (for *A. victrix*) to hyperparasitoid females. Colonies were held in the laboratory at room temperature under a L16:D8 photoperiod.

For the experiments, hyperparasitised mummies were individually collected in the rearing colonies, and kept as groups of 100 in a cage with a vial of sugar water as a food source at 20 ± 1 °C, and $75 \pm 10\%$ r.h., under a L16:D8 photoperiod. Males were added to ensure that at emergence the females had access to mates. From these cages 1–

6-day-old females were taken for use in the bioassays. These hyperparasitoids will live for more than 1 month under these experimental conditions (Christiansen-Weniger, 1992; Chow & Mackauer, 1996, R. Buitenhuis, unpubl.). The females were therefore not time-limited.

To obtain parasitised aphids and mummies for the biossays, third-instar aphid nymphs were exposed to parasitism by 3–5-day-old, mated *A. nigripes* females for a 24-h period. Parasitised aphids were then reared at 20 ± 1 °C and 75 ± 10% r.h., under a L16:D8 photoperiod. Based on the embryonic and larval developmental times of *A. nigripes* at 20 °C (Paré et al., 1979), third instar larvae in living aphids and prepupae in mummified aphids were obtained 5 and 8 days following parasitisation, respectively. In the text, these hosts are referred to as parasitised aphids and mummies.

Olfaction

Experimental set-up. Tests were carried out at room temperature (20–22 °C) in a Y-tube olfactometer (3.6 cm diameter, 30 cm long arms, distance to the junction of the arms 17.5 cm). For each arm, air was pumped through activated charcoal, humidified, adjusted to 4 cm s^{-1} (0.53 l min^{-1}) with an air flow meter (Omega© FL-1405), and led through a chamber containing the odour source. The air speed was chosen based on similar studies of primary and hyperparasitoids of aphids (Bouchard & Cloutier, 1985; Singh & Srivastava, 1987b). All the parts of the apparatus were connected using Tygon© tubing. The Y-tube was placed in a black box and its Y-end was oriented towards the one semi-transparent side, behind which a light source was placed (a circular Philips 22 W cool white fluorescent tube).

To ensure the functionality of the olfactometer, two types of tests were done. When both arms only carried clean air, hyperparasitoids (*A. victrix*, *A. suspensus*, and *S. aphidivorus*) chose each of them at the same frequency (χ^2-test, for all species, P>0.05; n>20). In the second test, males of the primary parasitoid *A. nigripes* more often chose the arm of the olfactometer with conspecific virgin females, as opposed to the clean air in the other arm ($\chi^2 = 7.6190$, P = 0.0058; n = 26 males).

Treatments. Treatments were chosen according to the aphid, and host quantities and chemical concentrations that were shown to be attractive to primary parasitoids and hyperparasitoids (Read et al., 1970; Bouchard & Cloutier, 1985; Siri, 1993).

(1) Single cues originating from all trophic levels.

From the first trophic level, we tested a clean potato seedling (Norland variety). A 15 cm high plant was washed, air dried, cut, and immersed in water sealed with Parafilm© to exclude any possible interference of volatiles from the cut edges. From the second trophic level, we tested potato aphids. One hundred aphids of all stages were collected in a gauze-covered container. In addition, we tested honeydew that had been collected according to the method of Bouchard & Cloutier (1984) (40 mg dried honeydew dissolved in 150 µl distilled water). Finally, from the third trophic level, we tested parasitised aphids, mummies, or female *A. nigripes*. For these treatments, either 100 4–5-day-parasitised aphids, 100 newly (0–24 h) mummified aphids, or six 1–5-day-old virgin *A. nigripes* females were collected in a gauze-covered container. Odours were tested in single choice tests against air (pumped through activated charcoal and humidified). A dual-choice test was performed for *S. aphidivorus* to determine the preference for mummies vs. parasitised aphids.

(2) Complex cues.

Aphid and possible aphid-induced plant volatiles were tested using a potato seedling infested with 50 potato aphids, 2 days before the test. The attraction of the whole plant–host complex was tested with a potato seedling infested for 2 days with 25 healthy aphids, 25 parasitised aphids, and 25 mummies, obtained as previously described. Mummies were glued on the leaves with non-toxic Lepage© white glue before the experiment. To exclude the possibility that hyperparasitoids were attracted to uninfested plant odours, the plant–host complex was tested in a dual choice test against a clean plant (washed and air dried potato seedling).

Bioassay. Mated 1–6-day-old hyperparasitoid females were given an oviposition experience of 24 h the day before the test with 10 mummies and five live parasitised aphids on a potato leaf, to standardise their searching and parasitising experience before the test. The females were released individually into the Y-tube, and used only once. After 5 min, the position of the female was recorded. This period of time was shown to be sufficient for the majority of the females to make a choice. If a female was found more than halfway (15 cm) into one of the arms of the olfactometer, this was recorded as a choice. Females recovered before this point, and at or before the intersection of the olfactometer arms, were not considered to have made a choice. Effectively, in the experiment, females were either found at the end of the tube, or at the intersection. The Y-tube and the containers for the odour sources were washed with hot water and acetone, and air-dried between each treatment. For each experiment (single and dual choice), all treatments were tested in a random order over a 2-day period. In each treatment, five females per hyperparasitoid species were tested in a random order. This was repeated eight times for a total of 40 females per species per treatment.

Foraging behaviour

Experimental set-up. Observations of the influence of potential cues on the foraging behaviour of hyperparasitoid females were made on 'Norland' potato plants under fluorescent lightning. All plants were selected to have 10 leaves (numbered from the base to the top), the same height (20–25 cm) and roughly the same shape and leaf surface area. A protocol similar to that of Cloutier & Bauduin (1990) was designed in order to further compare the behaviour of primary parasitoids and hyperparasitoids on the same plant-aphid system.

Treatments. Each plant was randomly allocated to one of the following treatments: control (uncontaminated plant), aphids (plant infested with 100 aphids for 2 days), honeydew (plant infested with 100 aphids for 2 days, after which aphids and exuviae were removed with a paintbrush before the experiment), aphids + parasitised aphids (PA) (plant infested for 2 days with 50 aphids and 50 parasitised aphids) and plant–host complex (PHC) (aphids + parasitised aphids, and two mummies glued on the underside of leaves 4, 6, and 8). This density was chosen to ensure that females would not spend all their time investigating and ovipositing in the mummies, considering that one or two encounters with mummies on the release leaf would be enough to induce a potential change in behaviour. Parasitised aphids were marked on the abdomen with a non-toxic marker (Sharpie©) to distinguish them from unparasitised aphids during the observations. This did not seem to disturb the aphids or to change their behaviour.

Bioassay. Mated 1–6-day-old females were given an oviposition experience of 24 h the day before the test, individually in cages with a potato leaf with hosts (for *A. suspensus* and *D. carpenteri* two mummies, for *S. aphidivorus* two mummies and two parasitised aphids, and for *A. victrix* two parasitised aphids), before being used in the experiments.

At the beginning of a test, one female was released from a gelatine capsule on the upper side of leaf number 4. Her behaviour was observed with Observer© software (Noldus 1997, Version 3 for Macintosh) for 1 h, or until the female left the plant for more than 5 s. One plant was used for one female of each hyperparasitoid species. Hosts that were parasitised by a hyperparasitoid female were replaced after each observation.

The duration of the following behaviours was recorded: walking, resting, grooming, feeding, flying, examining (aphid, parasitised aphid, or mummy), and ovipositing (aphid, parasitised aphid, or mummy). Furthermore, the position of the female was recorded continuously by noting the leaf number and plant part (upper- or underside of the leaf, petiole, or stem).

The order in which the hyperparasitoid species were tested was randomised within treatments. Ten females of each species were tested per treatment. Because the treatment with the parasitised aphids and the plant–host complex were the same for *A. victrix*, this species was not tested on the plant with parasitised aphids but only on the plant–host complex.

From the timetable created by the Observer© software the following parameters were calculated. The total visit time was defined as the time spent on the plant from release to departure. The search time was defined as the time spent walking. It was subdivided between time spent on the upper and lower surfaces of the leaves. The handling time was defined as the total time that a female spent examining and parasitising hosts during the visit. Finally, the number of leaves visited was calculated.

Only females that had come into contact with the tested stimuli were used in the analysis. Observations where the females immediately left the plant after aphid defence were discarded from the analysis because these did not represent a comparable visit (maximum of two cases out of 10 for *S. aphidivorus*).

Statistical analysis

The results of the olfactometer experiment were analysed using a χ^2-test, as the number of females that chose a certain odour was never lower than five. Censored data (visit time and search time) were analysed per species with the LIFEREG procedure, with a log-normal error function. The number of leaves visited was analysed with General Linear Models (GENMOD), with a Poisson error function. The time spent on the upper- or underside of the leaves was compared with a paired t-test. The level of significance was $\alpha = 0.05$ and all data were analysed using the SAS program (SAS Institute, 1999).

Results

Olfaction

Overall, 82% (min. 60%, max. 97% in any comparison) of the females made a choice for either of the two olfactometer arms. However, statistical tests showed that there was no significant preference of the four hyperparasitoid species for any odour source (Figure 1A–C), except for *S. aphidivorus*, which showed a preference for the odour of parasitised aphids over that of mummies in the dual choice test ($\chi^2 = 4.5151$, $P = 0.0336$) (Figure 1B).

Foraging behaviour

The total visit time of females of all hyperparasitoid species was affected by the different plant treatments (LIFEREG *A. victrix*: $\chi^2 = 12.9934$, d.f. = 3, $P = 0.0047$; *A. suspensus*:

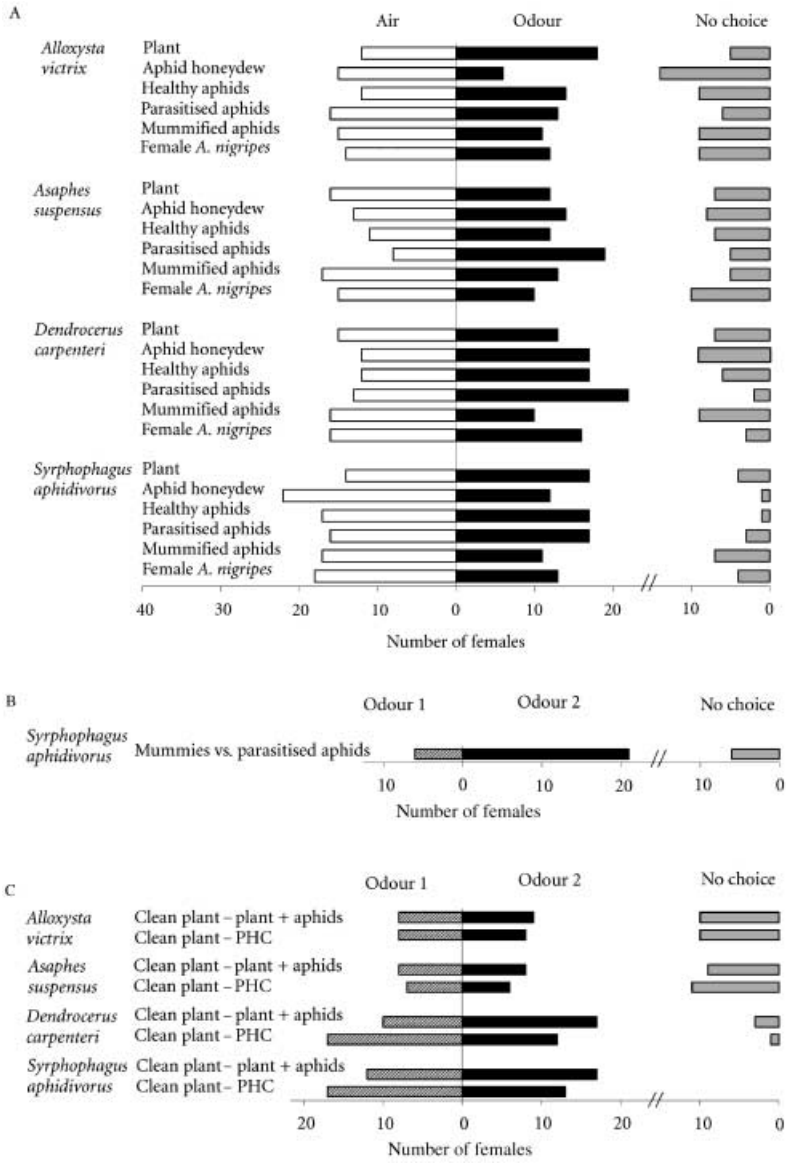

Figure 1 Preference of four aphid hyperparasitoid species for olfactory stimuli in a Y-tube olfactometer using the potato–*Macrosiphum euphorbiae*–*Aphidius nigripes* system. (A) Single choice test (odour vs. air). (B) Dual choice test of odours from the two hosts of *Syrphophagus aphidivorus*. (C) Dual choice test (odour 1 vs. odour 2); PHC = plant-host complex. Treatments indicated by an asterisk show significant differences (χ^2-test, $P<0.05$).

$\chi^2 = 11.9707$, d.f. = 4, $P = 0.0176$; *D. carpenteri*: $\chi^2 = 42.3305$, d.f. = 4, $P<0.0001$; *S. aphidivorus*: $\chi^2 = 47.0480$, d.f. = 4, $P<0.0001$) (Figure 2A). For all species, the females tended to spend more time on the plant following an increasing complexity of the stimuli.

The total visit time (Figure 2A) was divided into three categories of behaviours (Figure 2B–D): search time, time spent with hosts (examining and ovipositing into parasitised aphids or mummies), and other behaviours (resting, grooming, flying, feeding, and examining healthy aphids).

The search time was influenced by different stimuli for all hyperparasitoid species (LIFEREG *A. victrix*: $\chi^2 = 16.0711$, d.f. = 3, $P = 0.0011$; *A. suspensus*: $\chi^2 = 16.0553$, d.f. = 4, $P = 0.0029$; *D. carpenteri*: $\chi^2 = 38.9377$, d.f. = 4, $P<0.0001$; *S. aphidivorus*: $\chi^2 = 36.8214$, d.f. = 4, $P<0.0001$) (Figure 2B). Female *A. victrix* searched longer on plants with aphids and on the plant–host complex than on the other plant treatments. The other three species searched longer on all treatments compared to the control. Female *D. carpenteri* searched for the longest time on plants with honeydew and the plant–host complex. The search time of *S. aphidivorus* females was significantly longer on plants with parasitised aphids as compared to the other treatments.

The long duration of visits of the three mummy-attacking hyperparasitoids on plants with their hosts were actually caused by the time spent with mummies (Figure 2C). *Asaphes suspensus* spent 60 ± 4% (mean ± SE) of the total visit time examining and parasitising mummies, *D. carpenteri*

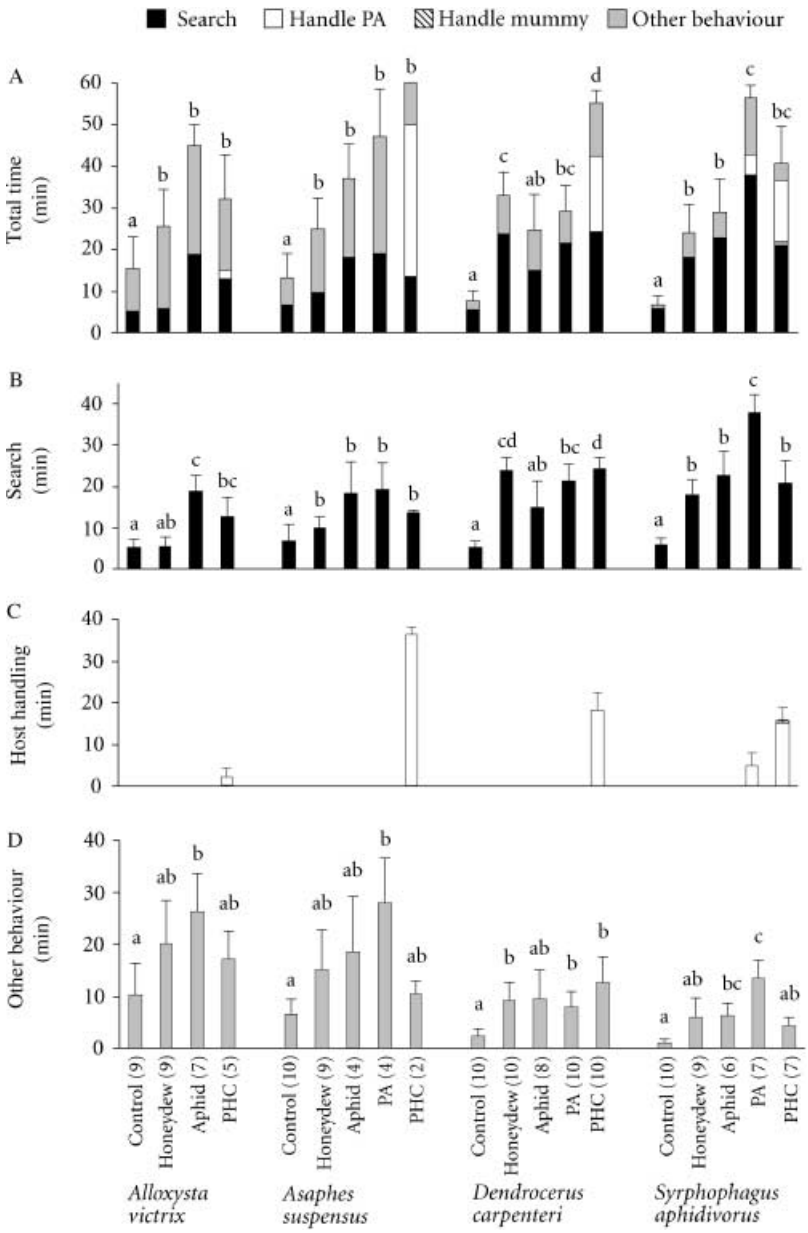

Figure 2 Effect of stimuli on host searching behaviour (mean + SE) of four aphid hyperparasitoid species searching on a potato plant. (A) Total visit time. (B) Search time. (C) Host attack. (D) Other behaviours (resting, grooming, flying, feeding, and examining aphids). Maximum observation time = 60 min. Between parentheses is indicated the number of females that were observed. Treatments are control (clean plant), honeydew (plant with *Macrosiphum euphorbiae* honeydew), aphid (plant infested with *M. euphorbiae*), PA (parasitised aphid, plant infested with *M. euphorbiae*, both healthy and parasitised by *Aphidius nigripes*), PHC (plant–host complex, as parasitised aphid treatment with additional *A. nigripes* mummies). Data were analysed per species with the LIFEREG procedure.

31 ± 21%, and *S. aphidivorus* 42 ± 18%. The time required to parasitise a mummy was very long (*A. suspensus* 1888 ± 204 s, *D. carpenteri* 462 ± 312 s, and *S. aphidivorus* 390 ± 138 s). In contrast, the time spent with parasitised aphids did not take such a substantial proportion of the total visit time (Figure 2C). *Alloxysta victrix* parasitised its hosts (larvae within live aphids) for only 7 ± 9% of the total visit time, and *S. aphidivorus* 10 ± 15% (PA) and 2 ± 2% (PHC). The time required to parasitise a host within a parasitised aphid was only 96 ± 18 s for *A. victrix* and 102 ± 48 s for *S. aphidivorus*. When hosts were present, a significant proportion of females stayed on the plant for the whole duration of the experiment (1 h; *A. suspensus* 100%, *A. victrix* 20%, *D. carpenteri* 70%, *S. aphidivorus* (PA) 71%, *S. aphidivorus* (PHC) 43%).

The number of leaves that was visited was generally small compared to the number of leaves available (10). There were differences in the number of leaves visited between species and between some treatments (two-way GENMOD; treatment $\chi^2 = 11.43$, d.f. = 4, P = 0.0221; species $\chi^2 = 41.48$, d.f. = 3, P<0.0001; treatment × species $\chi^2 = 30.87$, d.f. = 11, P = 0.0012) (Table 1). In general, *D. carpenteri* visited more leaves than the other three species. For each species, the differences in the number of leaves visited between the

Table 1 Number of leaves (mean ± SE) visited and total time spent on the upper and under leaf sides by four aphid hyperparasitoids searching on differently treated potato plants. Treatments are control (clean plant), honeydew (aphids removed from plant previously infested with *Macrosiphum euphorbiae*), aphids (plant infested with *M. euphorbiae*), parasitised aphids (plant infested with *M. euphorbiae* aphids, both unparasitised and parasitised by *Aphidius nigripes*), PHC (plant–host complex, as parasitised aphids plus *A. nigripes* mummies)

Species	Treatment	n[a]	No. leaves visited[b]	Total time on upper surface	Total time on under surface
Alloxysta victrix	Control	10	1.9 ± 0.4 a	188 ± 68	122 ± 66
	Honeydew	9	1.7 ± 0.4 a	206 ± 72	117 ± 50***[c]
	Aphid	7	3.3 ± 0.9 b	463 ± 93	495 ± 136
	PHC	5	2.0 ± 0.8 ab	204 ± 71	538 ± 195
Asaphes suspensus	Control	10	2.2 ± 0.9 a	187 ± 100	156 ± 99
	Honeydew	9	1.6 ± 0.2 a	342 ± 114	174 ± 37
	Aphid	4	3.5 ± 1.6 a	505 ± 187	464 ± 219
	Parasitised aphid	4	2.5 ± 1.0 a	599 ± 216	414 ± 170
	PHC	2	1.5 ± 0.5 a	320 ± 28	416 ± 92
Dendrocerus carpenteri	Control	10	3.0 ± 0.5 a	168 ± 37	109 ± 29
	Honeydew	10	6.5 ± 0.9 b	668 ± 111	576 ± 69
	Aphid	8	2.7 ± 1.0 a	485 ± 175	330 ± 163
	Parasitised aphid	10	5.2 ± 0.9 b	707 ± 146	429 ± 109
	PHC	10	6.0 ± 1.0 b	723 ± 107	599 ± 54
Syrphophagus aphidivorus	Control	10	1.4 ± 0.3 a	233 ± 100	100 ± 100
	Honeydew	10	2.5 ± 0.5 ab	604 ± 179	403 ± 69
	Aphid	6	3.2 ± 1.1 ab	567 ± 405	600 ± 197
	Parasitised aphid	7	4.4 ± 0.7 b	1073 ± 235	894 ± 160
	PHC	7	3.6 ± 0.9 b	405 ± 123	630 ± 165

[a]Number of females observed.
[b]Data were analysed with a GLM using a Poisson error function. Within species in the same column, means with the same letter do not differ significantly (P>0.05).
[c]Significant difference between time spend on upper- and underside of the leaf (paired t-test).

treatments are similar to the results for search time. *Asaphes suspensus* visited an equal number of leaves in each treatment, *A. victrix* visited more leaves on the plant with aphids, *D. carpenteri* visited more leaves on the honeydew, parasitised aphid, and plant–host complex treatments, and *S. aphidivorus* visited more leaves on the parasitised aphid and plant–host complex treatments compared to the control.

After release, female hyperparasitoids mainly explored the plant by walking. Females were only occasionally observed to use short flights to move between the leaves (1.1 ± 0.2 SE flights female^{-1} observation^{-1}). Females searched both sides of leaves, often alternating rapidly between the upper- and undersides. The time allocated to searching on the upper and lower surfaces of leaves did not differ significantly for any species or treatment, except for *A. victrix* (Table 1), which searched longer on the upper than the lower surface of leaves on honeydew-contaminated plants (paired t-test; $t_8 = 4.59$, $P = 0.0018$). When visiting different leaves, *A. suspensus* moved slightly upward on the plant in all treatments. *Alloxysta victrix* always moved to the highest leaves before taking off. *Dendrocerus carpenteri* and *S. aphidivorus* moved up and down on the plant in no clear pattern.

Discussion

Our results indicate that airborne olfactory cues are probably not essential cues in the host search of the four aphid hyperparasitoid species we studied, while cues that are encountered on a plant provide information that induces searching in most species.

Olfaction

Even though the hyperparasitoid females had been given an oviposition experience before the test, the odours of the potato–*M. euphorbiae*–*A. nigipes* system that we offered in the olfactometer were apparently not attractive to females. Although we cannot completely exclude that the negative results are an artefactual effect of the experimental set up, several arguments indicate that the Y-tube olfactometer is

relevant and that our results are valid. First, a similar set-up has been used successfully for aphid hyperparasitoids before (Read et al., 1970; Singh & Srivastava, 1987a,b; Siri, 1993). Second, pre-tests showed that the set-up was functional for primary parasitoids. *Aphidius nigripes* males were attracted to the odour of conspecific females. Third, we obtained one positive response from *S. aphidivorus*, which was attracted to the odour of live parasitised aphids vs. aphid mummies. However, we cannot explain why *S. aphidivorus* preferred the odour of live parasitised aphids to that of aphid mummies in the dual choice test, while in the single choice test it was neither attracted nor repelled by any of these odour sources.

Other studies, with similar set-ups, have reported varying results. *Alloxysta fuscicornis* (= *Charips brassicae*) was attracted to female primary parasitoids, but not to plant or aphid odours (Read et al., 1970). On the other hand, *Alloxysta pleuralis* responded to volatiles from various plants (Singh & Srivastava, 1987a,b). Finally, *A. victrix* was attracted to aphid-induced volatiles and a synthetic aphid alarm pheromone, and *D. carpenteri* responded to herbivore-induced volatiles, conspecific females and mummies, but neither species reacted to aphids, plants, or primary parasitoid females (Siri, 1993). The differences between these studies and ours might be explained by differences in the hyperparasitoid species that were tested, or may be due to differences in plant–aphid–primary parasitoid systems (oat–*Sitobion avenae*–*Aphidius uzbekistanicus*; Siri, 1993).

Foraging behaviour

Once they have arrived on a plant, aphid hyperparasitoid females are arrested by aphid and host-derived stimuli. Honeydew acted as a search stimulant for *A. suspensus*, *D. carpenteri*, and *S. aphidivorus*, but not for *A. victrix*. These results confirm that honeydew is a source of kairomones used in host finding by some hyperparasitoids (Budenberg, 1990; Buitenhuis et al., 2004b). These studies showed that aphid hyperparasitoids were arrested by honeydew on a filter paper disk, but this was never demonstrated on a whole plant. *Alloxysta victrix* is reported to be arrested by honeydew on a filter paper disk or a glass slide (Budenberg, 1990; Grasswitz, 1998; Buitenhuis et al., 2004b), which is in contrast with our findings on a plant. The observed indifference of *A. victrix* towards honeydew might be caused by the relatively young age of the *A. victrix* females that were tested (mostly 2 days old). More recent experiments have shown that this species has a pre-oviposition period of 2.1 days (R. Buitenhuis, unpubl.). Consequently, older females of this species might be more stimulated to search and might show different behaviour.

As could be expected, *S. aphidivorus* females spent more time searching on plants with parasitised aphids than on plants with unparasitised aphids. However, this was not observed on plants with mummies (plant–host complex). This is curious because, of the two hosts, mummies are reported to be the preferred and most suitable (Kanuck & Sullivan, 1992; Buitenhuis et al., 2004a). Perhaps the different proportions of parasitised aphids and mummies in the plant treatments had an influence on the females' perception of the patch. Further study will have to determine how in this species oviposition in one of the two hosts influences searching time.

The presence of hosts prolonged visit time in most cases, an effect that would probably be even stronger if the females were allowed to remain on the plant for more than 60 min. This increase in visit time was due to the long handling times of mummies (6 ± 2 to 32 ± 3 min) for *A. suspensus* and *D. carpenteri*. A longer search following successful oviposition (success-motivated search) could not be demonstrated in this experiment, but might be if females could be observed up to the point that they left the plant.

Influence of host stage and host range

There were no differences between species that corresponded to the host stage (mummy vs. parasitised aphid). We found that airborne direct cues from the host were not detected by olfaction. Therefore, to find hosts from a distance these hyperparasitoids would have to rely on indirect cues, which are the same for both hosts. In contrast, direct contact cues from the host probably play a greater role in the host-acceptance phase, where potential hosts are recognised by contact chemicals or by ovipositor probing (Christiansen-Weniger, 1992, 1994; Siri, 1993; Grasswitz & Reese, 1998; Grasswitz, 1998).

Another potential determinant of searching behaviour is the host range. Generally, the use of cues can be transposed on a specialist–generalist continuum: from intense and specific through weak and non-specific to the absence of cue use (Vet & Dicke, 1992). Similarly, the four tested hyperparasitoids ranged from one relatively host-specific species (*A. victrix*, attacking parasitoids of only one genus) to three species with a very large host range (*D. carpenteri*, *A. suspensus*, and *S. aphidivorus*, attacking a wide variety of genera) (van den Bosch, 1981; Höller et al., 1993; Brodeur, 2000). However, in this study, no differences between hyperparasitoid species could be observed that corresponded to host range.

Differences between trophic levels

Do primary parasitoids and hyperparasitoids use the same host searching strategy? For several aphid primary parasitoids, attraction to olfactory cues from plants, plant–aphid complexes, aphids (Powell & Zhang, 1983; Bouchard & Cloutier, 1985; Wickremasinghe & van Emden,

1992; Reed et al., 1995; Du et al., 1996; Vaughn et al., 1996; Du et al., 1997; Storeck et al., 2000; Völkl, 2000), honeydew (Bouchard & Cloutier, 1985), and aphid sex pheromone (Powell et al., 1998; Glinwood et al., 1999) have been reported. On a plant, honeydew, aphids, aphid sex pheromone, and honeydew-collecting ants arrest primary parasitoid females and induce them to search (Ayal, 1987; Cloutier & Bauduin, 1990; Powell et al., 1998; Völkl, 2000).

We designed this study to give a realistic comparison between the behaviour of aphid hyperparasitoids and the host search behaviour of the primary parasitoid *A. nigripes* (Bouchard & Cloutier, 1985; Cloutier & Bauduin, 1990) on the same potato–*Macrosiphum euphorbiae* system. In contrast to *A. nigripes*, which was attracted to the odours of several aphid species and to aphid honeydew (Bouchard & Cloutier, 1985), none of the four hyperparasitoids was attracted to olfactory cues. On the other hand, there were similarities in the search behaviour on a plant of the primary parasitoid and hyperparasitoids. *Aphidius nigripes* showed longer residence and searching times, visited more leaves and spent more time per leaf in response to honeydew and aphids (Cloutier & Bauduin, 1990). This arrestment and search stimulation was also found in the hyperparasitoid species. Not all hyperparasitoids were arrested by honeydew, in contrast to what was found for *A. nigripes*. Both upper and lower leaf surfaces were searched equally by most hyperparasitoids, in contrast to *A. nigripes*, which searched more on the lower leaf surface, where it was more likely to find *M. euphorbiae* aphids.

In summary, our study suggests that aphid hyperparasitoids may not resemble primary parasitoids in their attraction to olfactory stimuli, but it demonstrates that their behaviour on a plant shows several similarities, although this depends on the hyperparasitoid species in question. There are two non-exclusive explanations for the differences between primary parasitoids and hyperparasitoids. First, many of the cues that are direct and reliable for primary parasitoids are indirect cues for hyperparasitoids and therefore less reliable. First, the presence of aphids on a plant, a reliable cue for primary parasitoids, does not guarantee the presence of suitable parasitised aphids to hyperparasitoids. Secondly, compared to primary parasitoids, hyperparasitoids generally have a broader host range (Gordh, 1981; Sullivan, 1987; Sullivan & Völkl, 1999, but see van den Bosch, 1981 and Brodeur, 2000). Vet & Dicke (1992) hypothesised that, contrary to specialists, the use of kairomones by generalists should be weak and non-specific, or could even be impossible because the great diversity of potentially useful chemical information would generate a physiological constraint on sensory processing, and common chemical components would be very limited. The hyperparasitoids tested here have been reported on many different plants and aphids (e.g., Gutierrez & van den Bosch, 1970; Sullivan & van den Bosch, 1971; Johnson et al., 1979; Mertins, 1985; Thiboldeaux et al., 1987; Höller et al., 1993; Müller et al., 1999). In the absence of common, detectable cues it is therefore likely that aphid hyperparasitoids search mainly in the habitat where they are born, or select a habitat at random, and that search is induced by contact stimuli on the plant.

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